EFFECTS OF ULTRAVIOLET RADIATION ON CYPRIDINA LUCIFERIN AND LUCIFERASE

AURIN M. CHASE AND ARTHUR C. GIESE[1]
Physiological Laboratory, Princeton University, New Jersey

EIGHT FIGURES

Harvey found ('25) that in the presence of oxygen a luminescent mixture of a crude aqueous extract of luciferin and luciferase from Cypridina becomes dark after brief irradiation with a concentrated beam from a carbon arc. He demonstrated that this effect was not upon the luciferase but upon the luciferin, and attributed it to a rapid photochemical oxidation. He showed by the use of suitable filters that the effective wavelengths were between λ 4600 and 3800 A. Wavelengths longer than λ 4600 A became effective in the presence of certain photosensitizing dyes (Harvey, '26). The stability of luciferin and luciferase to ultraviolet may be only apparent and due to absorption of these radiations by impurities present in the crude extract. The partial purification of Cypridina luciferin described by Anderson ('35) removes most of the color and greatly increases the purity, while prolonged dialysis of luciferase produces an almost colorless solution and causes precipitation of some of the protein impurities. Reinvestigation of the effects of radiations—particularly the ultraviolet—on luciferin and luciferase prepared relatively free of strongly absorbing materials therefore appeared desirable, especially as the data might provide additional information as to the nature of these compounds.

Since luciferin and luciferase differ chemically, the experiments on each will be treated separately, the first section of the paper dealing with luciferin and the second with luciferase.

MATERIALS AND METHODS

Luciferin from Cypridina was extracted and carried through one cycle of purification by the procedure described by Anderson ('35). For each day's work 1.0 cc. of the butyl alcohol solution of luciferin was evaporated in vacuo and the residue was dissolved in 1.0 cc. of 0.1 *N* HCl which was subsequently kept in an ice water bath. A 0.03 cc. sample of this HCl-luciferin stock solution, dissolved in 10 cc. of 0.1 *M*, pH 6.7 phosphate buffer, was used for each test.

[1] Rockefeller Foundation Fellow.

An almost colorless, highly active luciferase solution was obtained by dialysis of 5 gm. of powdered Cypridina in 100 cc. of water, in the manner described previously (Giese and Chase, '40). The Cypridina used were from a lot that gave strong luminescence upon moistening since it was found that lots of Cypridina which gave weak luminescence contained a relatively small amount of active luciferase. For studies of the effects of ultraviolet light on luciferase, the dialysate was diluted fifty times in preparing the stock solution of the enzyme, in order that the rate of the luminescent reaction might not be too rapid for accurate measurement. In preparing the luciferase stock solution that was used for studying effects of irradiation on luciferin, on the other hand, the dialysed enzyme solution was diluted only ten times in order that the light should be emitted rapidly, since total light emitted represents luciferin concentration. For each experiment a 0.20 cc. sample of the appropriate luciferase stock solution was diluted to 10 cc. with water before being added to the luciferin.

The luciferase solution was added to the luciferin after a constant interval from the time the latter had been dissolved in buffer. For experiments on luciferin this interval was 5 minutes; for those on luciferase, 2 minutes. This interval must be maintained constant, since spontaneous non-luminescent oxidation of luciferin occurs in the buffer, with a consequent decrease in light emission. The luminescence was measured in the same manner as in a previous study (Giese and Chase, '40).

A Hanovia Alpine lamp — a quartz mercury arc running on 3.5 amperes at 85 volts, D.C.— was used in some of the experiments. The spectrum of this arc includes lines from the infra red to the far ultraviolet. The intensity of the radiations between λ 2000 and 3000 A at a 10.5 cm. distance from the surface of the arc was determined with a tantalum photocell.[2] Relative intensities may be measured quite accurately in this way and a day to day variation of only 5% was found. The approximate intensity in absolute units was obtained by calibrating the photocell against Hanovia ultraviolet meter No. 478, which had itself been calibrated against a standard.[3] This value was found to be of the order of 30 ergs/mm.^{2}/sec.

For a study of the effects of the far ultraviolet on luciferin, a Braun Lifelite was used. This is a mercury resonance lamp in quartz with

[2] We are indebted to Dr. H. C. Rentschler, Director of Research at the Westinghouse Electric and Manufacturing Co., for supplying the photocell and the Sterilamp for this work.

[3] We are indebted to the Hanovia Chemical and Mfg. Company for the loan of the ultraviolet meter and to Dr. W. T. Anderson of the Hanovia Company for calibration and instructions on the use of the meter.

about 85% of its photic energy at λ 2537 A (Leighton and Leighton, '35). Solutions were irradiated in a flat-bottomed quartz flask at a distance of 2 cm. from the center of the lamp, whose absolute intensity (λ 2000–3000 A) at 6 cm. distance was of the order of 70 ergs/mm.²/sec.

For studying the effects of the far ultraviolet on luciferase, a Westinghouse mercury resonance Sterilamp,[2] immersed in water, was used. The luciferase solution, in the flat-bottomed quartz flask, was irradiated in a water-bath of 25°C., 3.5 cm. above the lamp, while being shaken

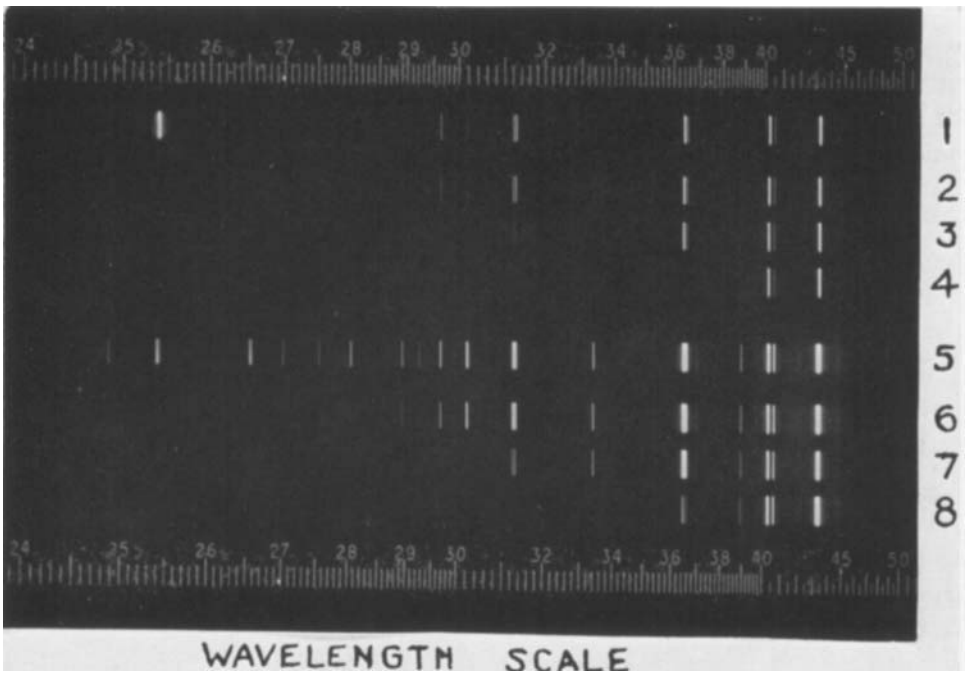

Fig. 1 Transmission of the mercury arc spectra of the Hanovia Alpine lamp and the Braun Lifelite lamp by three glass filters. (1) The emission spectrum of the Braun Lifelite with no filter present, (2) with the GG13 filter, (3) G86-B filter and (4) pyrex filter. (5) The emission spectrum of the Hanovia Alpine lamp. (6) With the GG13 filter, (7) G86-B filter, (8) pyrex filter.

gently. The intensity of the radiations between λ 3200 and 2000 A, measured by the Hanovia ultraviolet meter, at the distance used in this research, was about 30 ergs/mm.²/sec.

Since the mercury arc spectrum extends from the infra red to the far ultraviolet, it was necessary to limit the radiations with filters. A GG13 Jena glass, a G86-B Corning glass, and a thin piece of pyrex were used for this purpose. The transmission spectra of these filters are indicated in figure 1, which is printed from a photographic plate on which the

emission spectra of the two arcs were photographed, with and without the filters.[4] The Jena GG13 filter absorbs radiations of wavelength shorter than λ 3700 A, transmitting mainly the visible portion of the spectrum. The Corning G86-B filter absorbs radiations of wavelength shorter than λ 3000 A, transmitting the visible and near ultraviolet. The particular pyrex used transmits radiations of λ 2800 A and longer, therefore transmitting the visible, the near ultraviolet, and the long wavelength end of the far ultraviolet.

Ultraviolet absorption spectra of solutions were measured with a recording spectrophotometer (Harrison and Bentley, '40). Since the absorption spectrum of luciferin solutions does not remain constant during exposure to air (Chase, '40 a) this instrument was particularly suitable because of the relatively short time required to obtain an absorption curve. For these absorption measurements the concentration of the luciferin was about 100 times as great as that used for a luminescence determination. Absorption measurements of luciferase were made on the undiluted dialysed enzyme solution. The optical depth of the absorption cell was 10 mm.

I. Irradiation of luciferin

Effects of visible light. When luciferin solutions are irradiated with the Alpine lamp, luminescence appearing on addition of the enzyme is found to be much less than that of controls. However, when the radiations are filtered by the GG13 glass which transmits only the visible, the luciferin is not affected, and the luminescence obtained after irradiation is entirely comparable to that of the unirradiated control. This result is shown in figure 2, and indicates that visible light does not affect luciferin in the absence of colored impurities.

When eosin, fluorescein, or riboflavin is present in a luciferin solution during irradiation with visible light, a marked effect on the luciferin occurs. Typical experiments, given in figure 3, show that a dosage of 3 minutes of irradiation through the GG13 filter in the presence of one of these sensitizers reduces the total subsequent luminescence to less than one-half. Examination of the curves, however, discloses a qualitative difference between those for the two dyes and those for riboflavin. In the former case a dim luminescence continues for some time after the rapid light emission has ceased. The control curve also shows bright luminescence followed by prolonged dim light emission. In the case of

[4] We wish to thank Dr. Alfred L. Loomis for loaning a Jobin Yvon quartz spectrograph from the Loomis Laboratory at Tuxedo Park, New York.

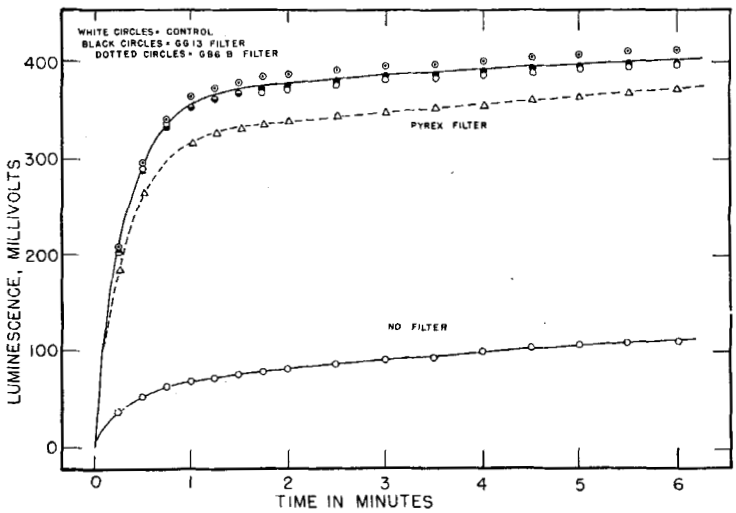

Fig. 2 Luminescence observed on adding luciferase to solutions of purified luciferin that have been irradiated for 2 minutes with the Hanovia Alpine lamp without any filter and with three glass filters whose transmission include the visible spectrum and extends to different points in the ultraviolet as indicated in figure 1. Wavelengths longer than λ 3000 A produce no effect upon purified luciferin since the luminescence measured is the same as that of the control, within the error of measurement. Wavelengths between λ 3000 and 2800 A cause some inhibition of luminescence and the unfiltered radiation of the arc has a great effect upon the luciferin, reducing luminescence to about 25% of that of the control.

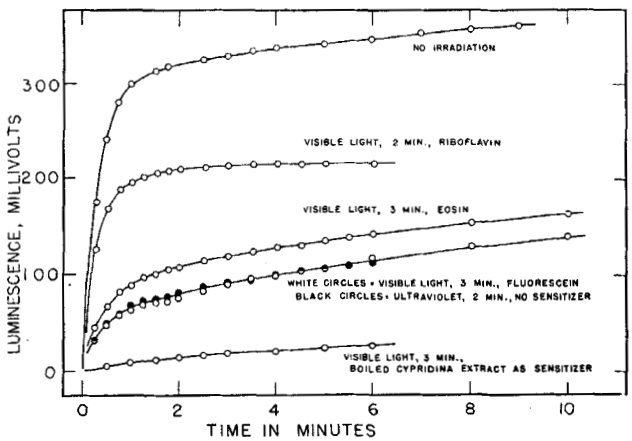

Fig. 3 Luminescence curves measured after irradiation of purified luciferin by visible light in the presence of eosin, fluorescein, riboflavin and boiled crude extract of Cypridina. All these substances sensitize luciferin to visible light. In the luminescence following irradiation of luciferin in presence of riboflavin practically no light emission occurs after 2 minutes.

riboflavin, however, the light emission is at first rapid and then ceases almost entirely.[5] In figure 4 this effect is strikingly brought out.

It is possible to interpret the difference in action between eosin and fluorescein on the one hand and riboflavin on the other in the following way. Anderson ('36) has shown that when luciferin is dissolved in buffer and exposed to air for various periods of time before the addition of luciferase, the amount of light rapidly emitted varies inversely with the exposure. The total light emitted is, however, approximately the same in all cases. To explain these results he has postulated the spontaneous formation of a reversibly oxidized compound when luciferin is

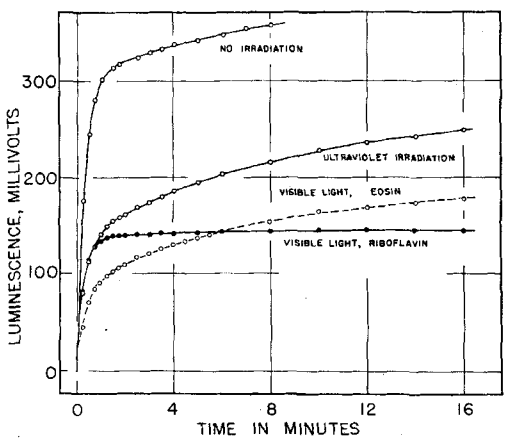

Fig. 4 Typical luminescence curve on adding luciferase to luciferin solutions which have been irradiated for 3 minutes with visible light in the presence of a 0.00075% concentration of riboflavin. Although an initial bright luminescence occurs, there is practically no light emission after 2 minutes.

The luminescence curves obtained after irradiation of luciferin with visible light when eosin is present, and after ultraviolet irradiation with no sensitizer present, both show not only a bright luminescence during the first 2 minutes, but a dim luminescence as well, which persists for at least 16 minutes.

---

[5] This abolishment of the slow, dim luminescence could conceivably result from selective absorption by the riboflavin since it has a strong light absorption near the region of the maximum of Cypridina luminescence. That this is not the case is shown by comparing the absorption spectrum of riboflavin of the concentration used with that of a similar concentration of fluorescein. Fluorescein absorbs more strongly in this region than does riboflavin, and yet a prolonged dim luminescence occurs after irradiation of luciferin with visible light in presence of fluorescein. The abolishment of the secondary dim luminescence by the riboflavin is, therefore, a real effect. The total measurable luminescence is of course decreased by the presence of dyes in the luminescent solution. This filtering effect can be evaluated by surrounding the reaction vessel with another glass vessel which contains the dye in a concentration calculated to be approximately equal to that present in the luminescent mixture. The apparent decrease in luminescence due to the filtering action of riboflavin in the solution was found to be not more than 20%, while the reduction of luminescence after irradiation in presence of riboflavin is very much greater than this.

exposed to air. The slow light emission he has attributed to the reduction and subsequent luminous oxidation of this compound in the presence of luciferase; the rapid light emission to rapid luminous oxidation of the unoxidized luciferin already present in the solution. When more than a minute or so elapses between solution of the luciferin and the addition of the luciferase both types of light emission occur and a compound curve describes the data. These two reactions are also evident after photochemical oxidation of luciferin. Apparently riboflavin not only sensitizes photochemical oxidation of luciferin but in addition reacts with some component of the system in such a way as to prevent the prolonged dim light emission which normally occurs. Determination of the exact part of the system affected, however, awaits further experimentation. Eosin and fluorescein apparently affect only the unoxidized luciferin, therefore acting simply as sensitizers.

Crude Cypridina extracts are sensitive to irradiation between λ 4600 and 3800 A (Harvey, '25). Since the purified luciferin is insensitive to these visible radiations, but is readily sensitized by dyes and by riboflavin, some natural sensitizer must be present in the crude material which sensitizes the luciferin to visible light. Chase ('40 b) found from visual observation that when boiled crude Cypridina extract was added to a glowing mixture of the purified luciferin and luciferase the luminescence was quickly quenched by glass-transmitted carbon arc radiations. This indicates that some component of the crude extract sensitizes the luciferin to either the near ultraviolet or the visible radiations. To determine if purified luciferin is sensitized to visible radiations by the crude Cypridina extract, 20% by volume of a boiled 1% crude extract was added to a sample of the purified luciferin and the mixture was irradiated with the Alpine lamp through a GG13 filter. As shown in figure 3, the total luminescence was very greatly reduced by sensitization with the crude extract.[6]

Effects of near ultraviolet radiations. When the G86-B filter was interposed between the Alpine lamp and the quartz flask containing the luciferin solution, and luciferase was subsequently added, the luminescence resembled that of the control as can be seen from the data plotted in figure 2. Since the G86-B filter transmits radiations of wavelengths

[6] Chase ('40 b) suggested that the dialyzable fraction of crude Cypridina aqueous extracts may contain a flavin and that this might account for some of the observed photosensitization. The concentration of flavin present must be very low, since measurements with the sensitive spectrophotometer designed by Shlaer ('38) show only a very small absorption maximum at λ 450 mμ, the maximum in the visible for riboflavin. The absorption spectrum in the ultraviolet was not determined with certainty because of the presence of other absorbing substances which mask the true flavin absorption bands.

longer than λ 3000 A, it is clear that the near ultraviolet does not affect luciferin.

If, instead of the G86-B filter, a piece of pyrex which transmitted radiations down to about λ 2800 A (see fig. 1) was used, the luciferin was found to be affected, but not so strongly as by unfiltered radiation. Therefore, the radiations of λ 2800 A and shorter wavelengths are apparently even more potent in their effect upon luciferin. Experiments were, therefore, performed with the short wavelength radiations from the Lifelite.

Effects of the far ultraviolet. Luciferin solutions given brief dosages with the Lifelite showed greatly decreased luminescence when mixed with luciferase. Characteristic data are given in figures 3 and 4.

The nature of the change in luciferin produced by irradiating it with ultraviolet light is not known. The effect is apparently confined to the unoxidized luciferin, for the prolonged dim light emission following the initial bright luminescence is very pronounced after irradiation with ultraviolet light. The change apparently occurs in the absence of molecular oxygen, for no luminescence was observed in a sample of luciferin solution irradiated after having been flushed for ½ hour with hydrogen purified by passage over hot platinized asbestos. Controls so treated were not affected by the procedure. The fact that the action of ultraviolet radiation upon luciferin appears to be independent of oxygen would indicate that the effect is different from the photochemical oxidation of luciferin by visible light previously described by Harvey ('25), where no quenching of luminescence occurred unless oxygen was present.

Absorption spectrum measurements. If far ultraviolet radiations affect luciferin, it should absorb this part of the spectrum. The absorption spectrum of a pH 6.8 solution of luciferin that had been carried through two cycles of purification is shown in figure 5. The inflections in this curve at about λ 2700 A and λ 3100–3200 A have been previously attributed to the luciferin (Chase, '40 a), but it must be remembered that there still is considerable impurity present which contributes to the visible yellow color of the solution and which may cause much of the ultraviolet absorption. The curve is presented not as the absorption spectrum of luciferin but simply to emphasize the high ultraviolet absorption of a relatively pure luciferin solution, such as used in these experiments. This absorption spectrum may explain the lack of effect of the visible and near ultraviolet wavelengths upon purified luciferin, since these portions of the spectrum are absorbed relatively slightly. The λ 2537 A, on the other hand, is strongly absorbed and its striking effect is, therefore, not surprising.

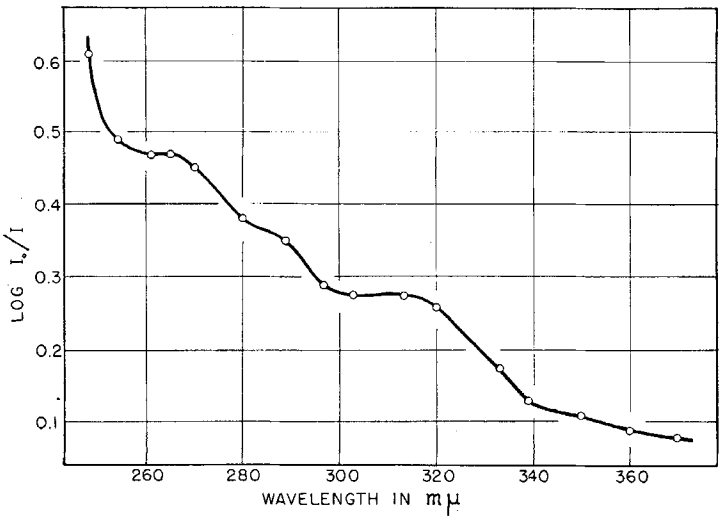

Fig. 5 Ultraviolet absorption spectrum of an aqueous solution of luciferin. Seven cubic centimeters of butyl alcohol solution of luciferin that had been carried through 2 cycles of purification were evaporated to dryness, re-dissolved in 1.0 cc. of 0.1 *N* HCl, diluted to 6 cc. with 0.25 *M* pH 6.8 phosphate buffer, and the HCl then neutralized with 1.0 cc. of 0.1 *N* NaOH. The depth of the absorption cell was 10 mm. While the inflections near λ 270 mμ and at λ 310–320 mμ are believed to be attributable to the luciferin, it is recognized that impurities are doubtless present, and the spectrum is presented merely to emphasize the high absorption of luciferin solutions at the shorter ultraviolet wavelengths.

II. Inactivation of luciferase by irradiation

When luciferase samples which had been irradiated with the Alpine lamp were added to luciferin, the rate of the luminescent reaction was less than that of the controls, due to inactivation of some of the enzyme; the decrease in rate varying with the dosage. Such inactivation occurred even when the medium was first flushed free of oxygen by bubbling purified hydrogen through the solution for ½ hour. Since the spectrum of the Alpine lamp includes far and near ultraviolet as well as visible light, it was necessary to determine which of these regions was inactivating the enzyme.

To study the effects of the visible spectrum the GG13 filter was interposed between the flask and the Alpine lamp. Even after long exposures there was no discernible effect upon the luciferase as determined by measuring the rate of the luminescent reaction. Typical data are given in column 3 of table 1. On the other hand when the unfiltered radiation of the arc was used, a 30 second dose caused a considerable decrease in the velocity constant (column 2 of table 1).

The possible effectiveness of the near ultraviolet was similarly tested by interposing the G86-B filter between the lamp and the cell. As the

TABLE 1

Luminescence (in millivolts) following irradiation of luciferase

Dosage min.	0	0.5	3.0	3.0	3.0	3.0	3.0	0	9.0
Filter	None	None	GG13	G86-B	GG13	GG13	GG13	None	GG13
Sensitizer	None	None	None	None	Ribo-flavin	Fluo-rescein	Eosin	Eosin	Eosin
% conc. of sensitizer	—	—	—	—	0.00075	0.00075	0.00075	0.00075	0.00075
Time in min.									
0.25	30	18	35	34	27	28	34	36	33
0.50	58	33	60	61	46	50	62	62	58
0.75	81	48	87	87	63	72	84	86	80
1.00	105	62	109	110	82	94	102	107	97
1.25	123	77	128	130	100	110	120	124	112
1.50	142	89	144	149	112	127	136	139	126
1.75	157	98	160	166	125	142	152	153	137
2.00	173	111	174	179	137	155	162	165	150
2.50	199	132	202	207	160	182	177	185	170
3.00	220	147	222	225	179	200	189	197	180
3.50	239	162	239	243	190	216	200	209	191
4.00	251	175	250	259	200	229	207	215	200
4.50	262	187	259	270	211	242	212	223	205
5.00	271	196	268	278	221	253	218	228	210
5.50	280	208	275	287	227	—	—	—	—
6.00	287	216	282	293	234	268	225	236	217
Dosage min.	0	0	2 min.	5 min.	0	5 min.	10 min.	0	5 min.
Filter	None	None	GG13	GG13	None	GG13	GG13	None	GG13
Sensitizer	None	Pina-cyanol[1]	Pina-cyanol	Bismarck brown	Bismarck brown	Methyl violet	Methyl violet	Methyl violet	Methy-lene blue
% conc. of sensitizer	—	0.000075	0.000075	0.00019	0.00019	0.00019	0.00019	0.00019	0.00019
Time in min.									
0.25	44	35	38	5	7	35	31	41	56
0.50	77	65	67	5	13	65	58	77	97
0.75	106	95	95	7	—	93	83	—	135
1.00	127	114	118	12	23	120	107	133	167
1.25	147	137	136	—	—	143	127	157	193
1.50	160	153	154	16	40	165	144	182	217
1.75	173	168	168	—	—	183	161	200	237
2.00	183	182	182	22	55	196	176	213	253
2.50	199	199	202	28	69	222	206	241	280
3.00	212	217	214	32	81	243	223	265	298
3.50	217	226	223	37	91	258	241	280	313
4.00	224	236	231	42	103	271	254	293	323
4.50	227	243	236	48	112	282	263	302	332
5.00	230	246	240	53	123	290	273	312	338
5.50	232	250	243	57	128	295	280	317	342
6.00	235	253	247	63	135	300	284	321	345

[1] 0.00075% pinacyanol completely inactivated luciferase both in the dark and in the light.

data in column 4 of table 1 show, no decrease in rate of the reaction occurred and it is, therefore, apparent that the near ultraviolet does not inactivate luciferase.

While visible light does not directly affect luciferase, it is possible that in the presence of a sensitizing dye it might do so. Consequently, luciferase was irradiated in the presence of various dyes whose concentration in the solution was 0.00075%, and the GG13 filter was interposed between the flask and the lamp. The luminescence measurements are given in columns 5 to 11 of table 1. Neither riboflavin, nor the two acid dyes, fluorescein and eosin, all of which sensitized luciferin, were effective in the case of luciferase. The observed reduction in total light is due to the filtering action of the added dyes which absorb a certain percentage of the light given off by the luminescent mixture.[7]

Of the basic dyes, methylene blue and methyl violet, both in 0.00019% concentration, were also found to be ineffective in causing the sensitization of luciferase to visible light. Pinacyanol in 0.00075% concentration caused inactivation of luciferase, with or without irradiation by visible light. In one-tenth this concentration it had no effect. Bismarck brown in 0.00019% concentration, of all the dyes tested, appeared to sensitize luciferase to irradiation with visible light. The data from the experiments with these acid and basic dyes are given in table 1.

Since neither the visible nor the near ultraviolet radiations affect luciferase, inactivation resulting from irradiation with the entire spectrum of the quartz mercury arc must be due to the far ultraviolet. Luciferase was, therefore, exposed for various periods of time to the radiations of the Sterilamp and was then added to luciferin in the manner previously indicated. The data from the luminescence measurements are given in table 2 and plotted in figure 6. It is quite clear that these radiations readily inactivate luciferase.

The curves of figure 6 can be described fairly adequately by the equation for a first order reaction (see Amberson, '21–'22), the rate being determined by the amount of luciferase present. It is, therefore, possible to compare the velocity constants of the curves and these should represent the relative amounts of luciferase remaining after the various dosages. In this comparison, a in the first order equation was taken as equal to 340 millivolts and the velocity constants were determined graphically by plotting log $(a - x)$ against time. When the logarithm of the per cent of luciferase remaining is plotted against the dosage, a practically straight line is obtained (see fig. 7). This indicates that at the very onset of irradiation inactivation of luciferase begins, and con-

[7] See footnote 5.

TABLE 2

Luminescence (in millivolts) following irradiation of luciferase

TIME[1] IN MINUTES	IRRADIATION TIME IN MINUTES						
	0.00	1.25	3.55	5.02	7.08	10.00	14.05
0.25	32	30	22	16	—	10	6
0.50	57	55	40	30	26	16	10
0.75	88	78	58	45	34	21	—
1.00	111	97	74	58	43	28	16
1.25	131	116	88	71	52	—	—
1.50	150	135	104	83	62	40	23
1.75	167	149	115	94	70	47	—
2.00	185	163	127	104	77	52	29
2.50	212	189	148	125	95	63	35
3.00	232	208	167	143	105	72	39
3.50	250	224	183	158	116	79	45
4.00	265	237	194	170	126	87	49
4.50	277	249	207	182	135	93	—
5.00	286	259	216	191	143	98	59
6.00	300	274	232	210	160	111	67
7.00	310	284	243	225	170	122	73
8.00	320	292	253	237	180	130	78
9.00	325	300	261	247	188	137	85
10.00	330	304	267	254	198	145	89
% luciferase left	100	83.6	58.8	46.8	32.6	18.4	7.8
Log % luciferase	2.00	1.92	1.77	1.67	1.51	1.26	0.89

[1] After mixture of luciferin and luciferase.

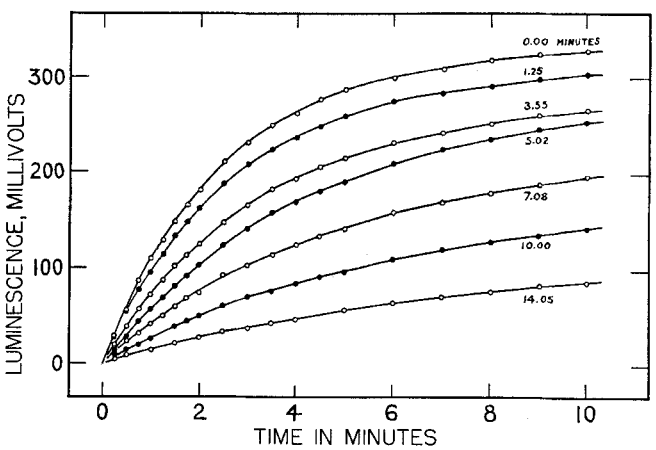

Fig. 6 Light emission curves obtained with a standard quantity of luciferin mixed with luciferase solutions that had been irradiated with a Westinghouse mercury resonance Sterilamp for various lengths of time. The velocity constant of the reaction decreases with increasing irradiation time, indicating inactivation of the luciferase.

tinues at a uniform rate, which is proportional to the concentration of active luciferase remaining. This relationship has sometimes been interpreted as indicating that a single quantum hit inactivates a given unit (Wyckoff and Rivers, '30; Gates, '34–'35).

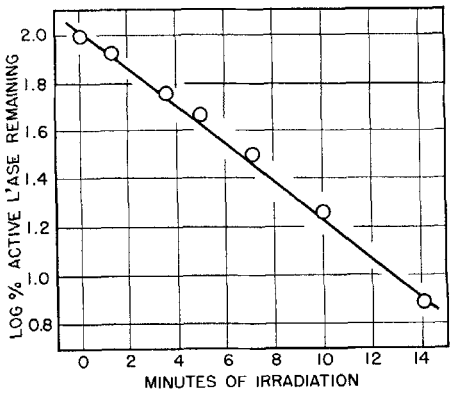

Fig. 7 Log per cent of the original luciferase activity remaining after various intervals of ultraviolet irradiation, plotted against time of irradiation. The data of table 2 were plotted as log (a – x) against time, taking a = 340 millivolts. For the first 5 minutes of the luminescent reaction the points could be fitted with straight lines, whose slopes, expressed as percentages of that of the control, are given at the bottom of table 2 and represent per cent active luciferase.

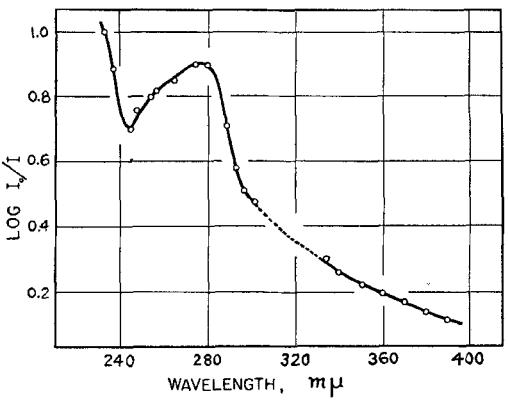

Fig. 8 Ultraviolet absorption spectrum of the luciferase stock solution used throughout these experiments, but some 2500 times more concentrated than when irradiated. The protein nature of the absorption curve is apparent. How much of the absorption is due to luciferase is not at present certain, since, although considerable protein lacking enzyme activity was removed by precipitation during dialysis, some probably remains in solution as impurity.

Light absorption by luciferase solutions. The dialysed luciferase solutions irradiated in these experiments are relatively opaque to the far ultraviolet and relatively transparent to the near ultraviolet and the visible, as shown by the absorption spectrum of figure 8. Absorption

maxima occur between λ 2800 and 2700 A and at wavelengths shorter than λ 2400 A, but there is very little absorption at wavelengths longer than λ 3000 A. This is typical of cytoplasmic proteins and resembles the spectrum found by Gates ('34–'35) for pepsin, but it must be remembered that while during the dialysis of the luciferase extract most of the visible color disappears and considerable protein is precipitated, nevertheless much protein impurity doubtless remains and probably contributes to the spectrum as measured. The absorption spectrum obtained however makes understandable the potency of the far ultraviolet in inactivating luciferase.

DISCUSSION

Both luciferin and luciferase are sensitive to far ultraviolet radiations when they are irradiated in the relative absence of impurities such as are present in a crude extract. These results seem to be in contradiction to the earlier statement (Harvey, '25) that visible light quenched luminescence of a crude Cypridina extract, but that the far ultraviolet was without significant effect. However, the crude extracts contain proteins and natural pigments, both of which absorb ultraviolet radiations readily and consequently act as protective filters for the luciferin and luciferase. It is possible that some inhibition of luminescence by the far ultraviolet may have occurred even in the case of the crude Cypridina extract, since actual measurements of luminescence were not made in the earlier study, but comparisons of luminescence intensity were made by eye. Finally, the possibility exists that the luciferin which results from Anderson's method of purification may not be the same compound which is present in the crude Cypridina extract.

Partially purified luciferin and dialysed luciferase are not affected by visible light, but in the presence of sensitizers luciferin is oxidized. Proteins do not appreciably absorb visible light, which consequently penetrates a crude extract readily. As has already been pointed out, a natural sensitizer is present in the crude extract. The luciferin is sensitized by this and since the solution transmits the radiations easily, the luciferin is affected.

A real difference between the effect of visible light on luciferin and the effect of ultraviolet light apparently exists, since the former effect is dependent on the presence of oxygen (Harvey, '25) while the effect of ultraviolet seems to occur in the absence of oxygen.

The protection of luciferase from injury by far ultraviolet light when impurities are present in the crude extract has a parallel in the resistance of cell enzymes to similar radiations when the entire cell is irradi-

ated. Thus Burge ('17) showed that the gelatin-liquefying activity of some bacteria is not significantly affected by a dosage of radiations which prevents subsequent division of the bacteria. The studies of Cook and Stephenson ('28) on bacteria, and of Oster ('34) on yeast, indicate that respiration continues at about the normal rate even after irradiation with dosages sufficient to prevent colony formation. However, enzymes freed from cellular material are known to be sensitive to ultraviolet radiations (Duggar, '36, chap. 37). While the sensitivity of various hydrolytic enzymes to ultraviolet light has been studied, this is the first time, to our knowledge, that an oxidative enzyme has been investigated in this way. The results on luciferase demonstrate that the two types of enzymes are similarly affected by these radiations.

Since partially purified luciferin and dialysed Cypridina luciferase extracts have such strong ultraviolet absorption it is not surprising that ultraviolet radiation affects them. The energy available from ultraviolet absorption is considerable, being about 114,000 cals per mole at λ 2500 A and about 95,000 cals per mole at λ 3000 A, and it is known to travel from the chromophoric group to other bonds (Carpenter, '40). The energy initially absorbed when a quantum of far ultraviolet radiation is taken up is adequate to disrupt most chemical bonds present in biological compounds (Pauling, '39). However, transfer of energy to distant bonds entails some dissipation and only the weaker ones are likely to be broken.

The energy in the visible portion of the spectrum is not only smaller (38,000–71,000 calories per mole) than in the ultraviolet, but is also much less readily absorbed by luciferin and luciferase. Prolonged exposure to visible radiations is therefore ineffective. In the case of luciferin, however, sensitizers make this energy available so that the molecule becomes modified—probably oxidized—and luminescence no longer occurs when luciferase is added. Luciferase, on the other hand, does not appear to be readily sensitized to the visible portion of the spectrum.

ACKNOWLEDGMENT

We wish to express our appreciation to Dr. E. N. Harvey for his interest and suggestions throughout this work, and to Dr. R. S. Anderson, Dr. E. G. Ball, and Dr. R. L. Garner for criticizing portions of the manuscript.

We are very greatly indebted to Dr. G. R. Harrison for allowing us to use the rapid recording spectrophotometer at the Spectroscopy Laboratory of the Massachusetts Institute of Technology.

SUMMARY

Cypridina luciferin purified by Anderson's method is not affected by irradiation with light of wavelengths longer than λ 3000 A, but is greatly affected by wavelengths shorter than this, particularly by the region λ 2800–2300 A. This effect appears to be independent of oxygen. Purified luciferin can be sensitized to visible light by adding crude boiled aqueous extract of Cypridina, or eosin, fluorescein or riboflavin. The last, in addition to its effect as a sensitizer, also has a specific effect upon the resulting reactions since the secondary prolonged dim light emission which ordinarily occurs on adding luciferase to a solution containing both luciferin and reversibly oxidized luciferin is totally abolished. The first part of the luminescence curve is not affected.

Irradiation of dialysed Cypridina luciferase with different parts of the spectrum shows that only the short wave ultraviolet (λ 2300–2800 A) causes inactivation. When per cent active luciferase remaining is plotted against irradiation time, a straight line describes the data.

Luciferin solutions exhibit increasingly great absorption toward the shorter wavelengths, with complete absorption below λ 2500 A and with maxima indicated at about λ 2700, 2900, and 3100–3200 A. The luciferase solutions show typical protein absorption with a maximum at about λ 2800 A and complete absorption at λ 2400 A. While it is recognized that impurities are probably still present in both the luciferin and luciferase solutions, the relatively high absorption of these solutions in the region λ 2400–2800 A is considered basis for the observed effects of ultraviolet radiations on both luciferin and luciferase.

The ineffectiveness of short wave ultraviolet radiation on crude Cypridina extracts is attributed to the presence in such extracts of impurities which protect the luciferin and luciferase by absorbing the shorter wavelengths. The ineffectiveness of visible light on purified luciferin solutions is explained by the absence from such solutions of an impurity which sensitizes luciferin to visible light.

LITERATURE CITED

AMBERSON, W. R. 1922 Kinetics of the bioluminescent reaction in Cypridina. I and II. J. Gen. Physiol., vol. 4, pp. 517–558.

ANDERSON, R. S. 1935 Studies in bioluminescence. II. The partial purification of Cypridina luciferin. J. Gen. Physiol., vol. 19, pp. 301–305.

———— 1936 Chemical studies on bioluminescence. III. The reversible reaction of Cypridina luciferin with oxidizing agents and its relation to the luminescent reaction. J. Cell. and Comp. Physiol., vol. 8, pp. 261–276.

BURGE, W. E. 1916 The mode of action of ultraviolet radiation in producing sterilization. J. Franklin Inst., vol. 182, pp. 264–265.

CHASE, A. M. 1940 a Changes in the absorption spectrum of Cypridina luciferin solutions during oxidation. J. Cell. and Comp. Physiol., vol. 15, pp. 159–172.

———— 1940 b Riboflavin and the photochemical oxidation of Cypridina luciferin. Amer. J. Physiol., vol. 129, pp. P332–P333.

COOK, R. P., AND M. STEPHENSON 1928 Bacterial oxidations by molecular oxygen. I. The aerobic oxidation of glucose and its fermentation products in its relation to the viability of the organism. Biochem. J., vol. 22, pp. 1368–1386.

DUGGAR, B. M. 1936 Biological effects of radiation. Vol. II, chap. 37. The effect of radiation on enzymes. McGraw Hill, New York.

GATES, F. L. 1934–1935 The absorption of ultra-violet radiation by crystalline pepsin. J. Gen. Physiol., vol. 18, pp. 265–278.

GIESE, A. C., AND A. M. CHASE 1940 The effect of cyanide on Cypridina luciferin. J. Cell. and Comp. Physiol., vol. 16, pp. 237–246.

HARRISON, G. R., AND E. P. BENTLEY 1940 An improved high speed recording spectrophotometer. J. Opt. Soc. America, vol. 30, pp. 290–294.

HARVEY, E. N. 1925 The inhibition of Cypridina luminescence by light. J. Gen. Physiol., vol. 7, pp. 679–685.

———— 1926 Further studies on the inhibition of Cypridina luminescence by light, with some observations on methylene blue. J. Gen. Physiol., vol. 10, pp. 103–110.

OSTER, R. H. 1934 Results of irradiating Saccharomyces with monochromatic ultra-violet light. I. Morphological and respiratory changes. J. Gen. Physiol., vol. 18, pp. 71–88.

PAULING, L. 1939 The nature of the chemical bond and the structure of molecules and crystals. Cornell University Press, Ithaca, New York.

SHLAER, S. 1938 A photoelectric transmission spectrophotometer for the measurement of photosensitive solutions. J. Opt. Soc. America, vol. 28, pp. 18–22.

WYCKOFF, R. W., AND T. M. RIVERS 1930 The effect of cathode rays upon certain bacteria. J. Exper. Med., vol. 51, pp. 921–932.